AMENDMENTS

In the Specification:

Please replace the entire section entitled "Brief Description of the Drawings" on page 3, lines 6 through 24:

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the sequence of HCV protease (SEQ ID NO: 69 and SEQ ID NO:70).

Figure 2 shows the polynucleotide sequence and deduced amino acid sequence of the clone C20c (SEQ ID NO: 71 and SEQ ID NO: 72).

Figure 3 shows the polynucleotide sequence and deduced amino acid sequence of the clone C26d (SEQ ID NO: 73 and SEQ ID NO: 74).

Figure 4 shows the polynucleotide sequence and deduced amino acid sequence of the clone C8h (SEQ ID NO: 75 and SEQ ID NO: 76).

Figure 5 shows the polynucleotide sequence and deduced amino acid sequence of the clone C7f (SEQ ID NO: 77 and SEQ ID NO: 78).

Figure 6 shows the polynucleotide sequence and deduced amino acid sequence of the clone C31 (SEQ ID NO: 79 and SEQ ID NO: 80).

B1

Figure 7 shows the polynucleotide sequence and deduced amino acid sequence of the clone C35 (SEQ ID NO: 81 and SEQ ID NO: 82).

Figure 8 shows the polynucleotide sequence and deduced amino acid sequence of the clone C33c (SEQ ID NO: 83 and SEQ ID NO: 84).

Figure 9 schematically illustrates assembly of the vector C7fC2OcC300C200.

Figure 10 shows the sequence of vector cf1SODp600 (SEQ ID NO: 85 and SEQ ID NO: 86).

Please replace the paragraph starting on page 6, line 23 with the following:

B1

The term "HCV protease" refers to an enzyme derived from HCV which exhibits proteolytic activity, specifically the polypeptide encoded in the NS3 domain of the HCV

genome. At least one strain of HCV contains a protease believed to be substantially encoded by or within the following sequence:

Arg Arg Gly Arg Glu Ile Leu Leu Gly Pro	10
Ala Asp Gly Met Val Ser Lys Gly Trp Arg	20
Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln	30
Gln Thr Arg Gly Leu Leu Gly Cys Ile Ile	40
Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln	50
Val Glu Gly Glu Val Gln Ile Val Ser Thr	60
Ala Ala Gln Thr Phe Leu Ala Thr Cys Ile	70
Asn Gly Val Cys Trp Thr Val Tyr His Gly	80
Ala Gly Thr Arg Thr Ile Ala Ser Pro Lys	90
Gly Pro Val Ile Gln Met Tyr Thr Asn Val	100
Asp Gln Asp Leu Val Gly Trp Pro Ala Ser	110
Gln Gly Thr Arg Ser Leu Thr Pro Cys Thr	120
Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr	130
Arg His Ala Asp Val Ile Pro Val Arg Arg	140
Arg Gly Asp Ser Arg Gly Ser Leu Leu Ser	150
Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser	160
Ser Gly Gly Pro Leu Leu Cys Pro Ala Gly	170
His Ala Val Gly Ile Phe Arg Ala Ala Val	180
Cys Thr Arg Gly Val Ala Lys Ala Val Asp	190
Phe Ile Pro Val Glu Asn Leu Glu Thr Thr	200
Met Arg (SEQ ID NO:1)	202

B1 Cont

Please replace the paragraph on page 8, starting on line 18 with the following:

TABLE 1: Alignment of Active Residues by Sequence

B2

Protease	His	Asp	Ser
HCV	CWTVYHGAG(SEQ ID NO:2)	DODLGWPAP(SEQ ID NO:3)	LKGSSGGPL(SEQ ID NO:4)
Yellow Fever	FHTMWHVTR(SEQ ID NO:5)	KEDLVAYGG(SEQ ID NO:6)	PSGTSGSPI(SEQ ID NO:7)
West Nile Fever	FHTLWHTTK(SEQ ID NO:8)	KEDRLCYGG(SEQ ID NO:9)	PTGTSGSPI(SEQ ID NO:10)
Murray Valley	FHTLWHTTR(SEQ ID NO.11)	KEDRVTYGG(SEQ ID NO:12)	PIGTSGSPI(SEQ ID NO:13)
Kunjin Virus	FHTLWHTTK(SEQ ID NO.14)	KEDRLCYGG(SEQ ID NO:15)	PTGTSGSPI(SEQ ID NO:16)

Please replace the paragraph on page 9, starting on line 9 with the following:

B3

TABLE 2: Alignment of Active Residues by Structure

Protease	His	Asp	Ser
S. griseaus A	TAGHC(SEQ ID NO:17)	NNDYGII(SEQ ID NO:18)	GDSGGSL(SEQ ID NO:19)
α-Lytic protease	TAGHC(SEQ ID NO:20)	GNDRAWV(SEQ ID NO:21)	GDSGGSW(SEQ ID NO:22)
Bovine Trypsin	SAAHC(SEQ ID NO:23)	NNDIMLI(SEQ ID NO:24)	GDSGGPV(SEQ ID NO:25)
Chymotrypsin	TAAHC(SEQ ID NO.26)	NNDITLL(SEQ ID NO:27)	GDSGGPL(SEQ ID NO:28)
Elastase	TAAHC(SEQ ID NO.29)	GYDIALL(SEQ ID NO:30)	GDSGGPL(SEQ ID NO:31)
HCV	TVYHG(SEQ ID NO:32)	SSDLYLV(SEQ ID NO:33)	GSSGGPL(SEQ ID NO:34)

B3 Cont

Please replace the paragraph on page 13 starting at line 11 with the following:

A presently preferred expression system employs the ubiquitin leader as the fusion partner. Copending application USSN 7/390,599 filed 7 August 1989 disclosed vectors for high expression of yeast ubiquitin fusion proteins. Yeast ubiquitin provides a 76 amino acid polypeptide which is automatically cleaved from the fused protein upon expression. The ubiquitin amino acid sequence is as follows:

B4

Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr
Leu Glu Val Glu Ser Ser Asp Thr Ile Asp Asn Val Lys
Ser Lys Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln
Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly
Arg Thr Leu Ser Asp Tyr Asn Ile Gln Lys Glu Ser Thr
Leu His Leu Val Leu Arg Leu Arg Gly Gly (SEQ ID NO:35)

Please replace the paragraph on page 21 starting at line 4 with the following:

In the absence of this protease activity, the HCV polyprotein should remain in its unprocessed form, and thus render the virus noninfectious. Thus, the protein is useful for assaying pharmaceutical agents for control of HCV, as compounds which inhibit the protease activity sufficiently will also inhibit viral infectivity. Such inhibitors may take the form of organic compounds, particularly compounds which mimic the cleavage site of HCV recognized by the protease. Three of the putative cleavage sites of the HCV polyprotein have the following amino acid sequences:

B5

Val-Ser-Ala-Arg-Arg // Gly-Arg-Glu-Ile-Leu-Leu-Gly (SEQ ID NO:36)

B5 cont

Ala-Ile-Leu-Arg-Arg // His-Val-Gly-Pro- (SEQ ID NO:88)

Val-Ser-Cys-Gln-Arg // Gly-Tyr- (SEQ ID NO:89)

Please replace the paragraph on page 24 starting at line 16 with the following:

First, DNA isolated from pSODCF1 was treated with BamHI and EcoRI, and the following linker was ligated into the linear DNA created by the restriction enzymes:

B6

GAT CCT GGA ATT CTG ATA AGA CCT TAA GAC TAT TTT AA (SEQ ID NO:37)

Please replace the paragraph on page 25 starting on line 1 with the following:

Three separate expression vectors, pcf1AB, pcf1CD, and pcf1EF were created by ligating three new linkers, AB, CD, and EF to a BamHI-EcoRI fragment derived by digesting to completion the vector pSODF1 with EcoRI and BamHI, followed by treatment with alkaline phosphatase. The linkers were created from six oligomers, A, B, C, D, E, and F. Each oligomer was phosphorylated by treatment with kinase in the presence of ATP prior to annealing to its complementary oligomer. The sequences of the synthetic linkers were the following:

B7

Name	DNA Sequence (5' to 3')
A	GATC CTG AAT TCC TGA TAA(SEQ ID NO:38)
B	GAC TTA AGG ACT ATT TTA A(SEQ ID NO:39)
C	GATC CGA ATT CTG TGA TAA(SEQ ID NO:40)
D	GCT TAA GAC ACT ATT TTA A(SEQ ID NO:41)
E	GATC CTG GAA TTC TGA TAA(SEQ ID NO:42)
F	GAC CTT AAG ACT ATT TTA A(SEQ ID NO:43)

Please replace the paragraph on page 27 starting on line 2 with the following:

B8

Clone C33c was isolated using a hybridization probe having the following sequence:

B8 Cont 5' ATC AGG ACC GGG GTG AGA ACA ATT ACC ACT 3' (SEQ ID NO:44)

Please replace the paragraph on page 27 starting on line 7 with the following:

B9 Clone 35 was isolated by screening with a synthetic polynucleotide having the sequence:

5' AAG CCA CCG TGT GCG CTA GGG CTC AAG CCC 3' (SEQ ID NO:45)

Please replace the paragraph on page 27 starting on line 20 with the following:

B10 Clone 7f was isolated using a probe having the sequence:

5'-AGC AGA CAA GGG GCC TCC TAG GGT GCA TAA T-3' (SEQ ID NO:46)

Please replace the paragraph on page 27 starting on line 24 with the following:

B11 Clone C20c is isolated using a probe having the following sequence:

5'-TGC ATC AAT GGG GTG TGC TGG-3' (SEQ ID NO:47)

Please replace the paragraph on page 28 starting on line 6 with the following:

Clone 8h was isolated using a probe based on the sequence of nucleotides in clone 33c.

B12 The nucleotide sequence of the probe was

5'-AGA GAC AAC CAT GAG GTC CCC GGT GTT C-3' (SEQ ID NO:48)

Please replace the paragraph on page 28 starting on line 11 with the following:

B13 Clone C26d is isolated using a probe having the following sequence:

5'-CTG TTG TGC CCC GCG GCA GCC-3' (SEQ ID NO:49)

Please replace the paragraph on page 30 starting on line 8 with the following:

B14 This vector contains a full-length HCV protease coding sequence fused to the FLAG sequence, Hopp et al. (1988) Biotechnology 6: 1204-1210. PCR was used to produce a HCV protease gene with special restriction ends for cloning ease. Plasmid p500 was digested with

EcoRI and NdeI to yield a 900 bp fragment. This fragment and two primers were used in a polymerase chain reaction to introduce a unique Bg1II site at amino acid 1009 and a stop codon with a SalI site at amino acid 1262 of the HCV-1, as shown in Figure 17 of WO/11089, published 4 October 1990. The sequence of the primers is as follows:

5' CCC GAG CAA GAT CTC CCG GCC C 3' (SEQ ID NO:50)

and

5' CCC GGC TGC ATA AGC AGT CGA CTT GGA 3' (SEQ ID NO:51)

After 30 cycles of PCR, the reaction was digested with BGlII and SalI, and the 710 bp fragment was isolated. This fragment was annealed and ligated to the following duplex:

B14 Cont

MetAspTyrLysAspAspAspAspLysGlyArgGlu
CATGGACTACAAAGACGATGACGATAAAGGCCGGGAG (SEQ ID NO: 52)
CTGATGTTTCTGCTACTGCTATTTCCGGCCCTCTAG (SEQ ID NO: 90)

The duplex encodes the FLAG sequence, and initiator methionine, and a 5' NcoI restriction site. The resulting NCoI/SalI fragment was ligated into a derivative of pCF1.

Please replace the paragraph on page 34 starting on line 4 with the following:

B15

This vector contains HCV sequence, which includes the wild-type full-length HCV protease coding sequence, fused at the 5' end to a SOD coding sequence. Two fragments, a 441 bp EcoRI/Bg1II fragment from clone 11b and a 1471 bp Bg1III/EcoRI fragment from expression vector P500, were used to reconstruct a wild-type, full-length HCV protease coding sequence. These two fragments were ligated together with an EcoRI digested pS356 vector to produce an expression cassette. The expression cassette encodes the ADH2/GAPDH hybrid yeast promoter, human SOD, the HCV protease, and a GAPDH transcription terminator. The resulting vector was digested with BamHI and a 4052 bp fragment was isolated. This fragment was ligated to the BamHI digested pAB24 vector to produce p650. p650 expresses a polyprotein containing, from its amino terminal end, amino acids 1-154 of hSOD, an oligopeptide -Asn-Leu-Gly-Ile-Arg- (SEQ ID NO:87), and amino acids 819 to 1458 of HCV-1, as shown in Figure 17 of WO 90/11089, published 4 October 1990.

Please replace the paragraph on page 34 starting on line 17 with the following:

Clone 11b was isolated from the genomic library of HCV cDNA, ATCC accession no. 40394, as described above in Example 3A, using a hybridization probe having the following sequence:

B14

5' CAC CTA TGT TTA TAA CCA TCT CAC TCC TCT 3' (SEQ ID NO:54).

This procedure is also described in EPO Pub. No. 318 216, Example IV.A.17.

Please replace the paragraph on page 34 starting on line 22 through page 35, line 12 with the following:

The vector pS3EF, which is a pBR322 derivative, contains the ADH2/GAPDH hybrid yeast promoter upstream of the human superoxide dimutase gene, an adaptor, and a downstream yeast effective transcription terminator. A similar expression vector containing these control elements and the superoxide dismutase gene is described in Cousens et al. (1987) Gene 61: 265, and in copending application EPO 196,056, published October 1, 1986. pS3EF, however, differs from that in Cousens et al. in that the heterologous proinsulin gene and the immunoglobulin hinge are deleted, and Gln_{154} of SOD is followed by an adaptor sequence which contains an EcoRl site. The sequence of the adaptor is:

B17

5' AAT TTG GGA ATT CCA TAA TTA ATT AAG 3' (SEQ ID NO:55)

3' AC CCT TAA GGT ATT AAT TAA TTC AGCT 5' (SEQ ID NO:56)

The EcoRI site facilitates the insertion of heterologous sequences. Once inserted into pS3EF, a SOD fusion is expressed which contains an oligopeptide that links SOD to the heterologous sequences. pS3EF is exactly the same as pS356 except that pS356 contains a different adaptor. The sequence of the adaptor is shown below:

5' AAT TTG GGA ATT CCA TAA TGA G 3' (SEQ ID NO:57)

3' AC CCT TAA GGT ATT ACT CAG CT 5' (SEQ ID NO:58)

pS356, ATCC accession no. 67683, is deposited as set forth below.

Please replace the paragraph on page 37 starting at line 4 with the following:

Four synthetic DNA fragments were annealed and ligated** together to create a EcoRI/SacI Yellow Fever leader, which was ligated to a EcoRI/SacI digested pGEM®-3Z vector from Promega®. The sequence of the four fragments are listed below:

YFK-1:

5' AAT TCG TAA ATC CTG TGT GCT AAT TGA GGT GCA TTG GTC TGC AAA TCG AGT TGC TAG GCA ATA AAC ACA TT 3' (SEQ ID NO:59)

YFK-2:

5' TAT TGC CTA GCA ACT CGA TTT GCA GAC CAA TGC ACC TCA ATT AGC ACA CAG GAT TTA CG 3'(SEQ ID NO:60)

YFK-3:

B18

5' TGG ATT AAT TIT AAT CGT TCG TTG AGC GAT TAG CAG AGA ACY GAC CAG AAC ATG TCT GAG CT 3' (SEQ ID NO:61)

YFK-4:

5' CAG ACA TGT TCT GGT CAG TTC TCT GCT AAT CGC TCA ACG AAC GAT TAA AAT TAA TCC AAA TGT GTT 3' (SEQ ID NO:62)